## REMARKS

Applicants submit this Corrected Amendment and Response to Office Action to correct a pagination error in the Amendment and Response filed November 17, 2006, which pagination error was noted in the Notice of Non-Compliant Amendment dated July 26, 2007. The remainder of this Remarks section is identical to the Amendment and Response filed November 17, 2006.

The Advisory Action indicated that the amendment filed July 18, 2006 would not be entered because Applicants "substantially" changed the scope of the claimed invention and therefore a new search and consideration of issues with respect to 35 USC §112 is required. Applicants do not believe that any new issues with respect to art or to 35 USC §112 are raised by this Amendment. Nevertheless, applicants would welcome the opportunity to respond in the event that the Examiner decides otherwise.

The Advisory Action also indicated that the Declaration of Dr. Timothy Edmunds would not be entered because "the Applicant failed to provide a showing of good and sufficient reasons why the affidavit ... is necessary and was not earlier presented." Applicants discussed the filing of such a Declaration with the Examiner during the March 1, 2006 interview. In fact, in the Interview Summary Record dated March 15, 2006, the Examiner expressly stated that "evidence indicating that production of a pharmaceutical useful glucocerebrosidase is independent of the mammalian cell type and inhibitor of carbohydrate processing used would be beneficial."

Nevertheless, subsequent to receiving the Advisory Action, Applicants discovered that the application may have become abandoned for failure to file a Notice of Appeal with the July 18, 2006 Response. Accordingly, Applicants submit herewith both a Request for Continued Examination and a Petition to Revive an unintentionally abandoned application pursuant to 37 CFR 1.137(b) and request that these remarks be considered and the requested amendment be entered upon the granting of such Petition.

Claims 60-62 are pending. Claims 60 and 61 have been amended. Claims 1-47 have been previously cancelled. Claims 48-59 and 63-72 have been previously withdrawn as a result of a restriction requirement and species election. The claims have been amended or cancelled without any intention to abandon the subject matter of those

claims as filed or later amended, but with the intention that claims of the same, greater, or lesser scope may be pursued in a continuing application.

Applicants acknowledge with appreciation the Examiner's withdrawal of the double patenting rejection and the claim rejections under 35 U.S.C. §102.

Claims 60 and 61 have been amended to clarify that the glucocerebrosidase that is recovered from a culture of mammalian cells treated with an inhibitor of carbohydrate processing has a higher number of exposed mannose residues than would glucocerebrosidase recovered from a culture of the same mammalian cells in the absence of such treatment. Support for this amendment can be found in the specification at page 28, lines 7-11. No new matter has been added.

***Priority***

Applicants have submitted herewith a new application data sheet to delete the priority claim to U.S. Application Number 07/289,589, filed December 23, 1988. This application now claims priority to December 22, 1989.

***Interview***

Applicants would like to thank the Examiner for the telephonic interview conducted on March 1, 2006, including his careful consideration of the application and helpful discussion of the issues raised in the Office Action. During the interview, the rejections and potential responses were reviewed, including the submission of a Declaration. Applicants believe that the claim amendments, remarks, and Declaration of Dr. Timothy Edmunds submitted herewith overcome the outstanding rejections thereby placing this case in condition for immediate allowance.

***Rejection of claims under 35 USC § 112, First paragraph – Written description***

Claims 60-62 were rejected under 35 USC § 112, first paragraph, as failing to comply with the written description requirement. In the January 19, 2006 Office Action, the Examiner stated that "the specification does not disclose a representative number of species or the relevant identifying characteristics that define a genus of any human enzyme having an activity which causes hydrolysis of a glucocerebrosidase."

In response, Applicants note first that they do not dispute that the specification teaches on page 6 lines 31-33 that glucocerebrosidase has an enzyme activity which causes hydrolysis of a glucocerebrosidase and it further states that "[t]his invention includes all enzymes having such activity...." However, the invention now claimed is much narrower. The amended claims do not encompass all enzymes having such activity. Rather, the amended claims refer to mammalian cells capable of expressing "human glucocerebrosidase." In Dr. Timothy Edmunds' Declaration submitted herewith, he states at paragraph 8 that there is currently believed to be only one human glucocerebrosidase and that the sequence of the human glucocerebrosidase gene, and the amino acid sequence it encodes, are well known in the art (EC 3.2.1.2.45). Thus, the glucocerebrosidase of the pending claims is limited to a single human protein of known amino acid sequence. It is not a genus of proteins. Applicants also refer the Examiner to the disclosure on page 2 of the Specification at lines 4-7 and the cited references, namely Sorge et al., Proc. Nat'l Acad. Sci., 7289 (1985) and Tsuji et al., J. Biol. Chem. 261:50 (1986) which indicate that the human glucocerebrosidase gene was cloned as early as 1985. In the Advisory Action, the Examiner acknowledged that these arguments were persuasive with respect to the scope of the "human glucocerebrosidase" of the claims.

However, the Examiner nevertheless maintained the rejection, asserting that "the specification provides no specific disclosure of which combination within the broad scope of a glucocerebrosidase produced by any mammalian cell exposed to any inhibitor of carbohydrate processing that acts to inhibit conversion of $Glc_3Man_9GlcNac_2$ to smaller species will comprise the requisite carbohydrate structure." In the Advisory Action, the Examiner further clarified the basis of his rejection by explaining that "the rejection does not assert that the genus 'mammalian cell' has not been described, but that the combination of mammalian cell and inhibitor of carbohydrate processing required to produce a glucocerebrosidase having at least two carbohydrate moieties each having a $Man_3$-$Man_9$ structure wherein such rGCR represents at least 50% of the rGCR has not been described."

Applicants submit herewith a Declaration of Dr. Timothy Edmunds containing experimental data demonstrating that a skilled artisan, following the teachings of the specification, can in fact control the glycosylation process in mammalian cell culture to

produce glucocerebrosidase containing a higher number of exposed mannose residues than glucocerebrosidase produced from the same cells in the absence of such treatment.

Specifically, Dr. Edmunds presents evidence that the skilled artisan could readily treat various mammalian cells capable of expressing human glucocerebrosidase with various inhibitors of carbohydrate processing that act to inhibit the conversion of $Glc_3Man_9GlcNac_2$ to smaller species to produce the glucocerebrosidase compositions of the claims.

As set forth in paragraphs 10-12 of Dr. Edmunds' Declaration, cultures of CHO cells capable of expressing human glucocerebrosidase were treated with four different inhibitors of carbohydrate processing encompassing three different classes: castanospermine and deoxynojirimycin (both glucosidase inhibitors); deoxymannojirimycin (a mannosidase I inhibitor); and swainsonine (a mannosidase II inhibitor). Glucocerebrosidase was purified from the culture medium, the samples were treated with Endoglycosidase H ("Endo H") (an enzyme that removes oligomannose type and hybrid type sugar structures but would have no effect on glucocerebrosidase containing complex sugars), and the samples were run on an SDS PAGE gel. As demonstrated in Figure 1 of Exhibit B of Dr. Edmunds' Declaration, upon treatment with Endo H, there was no change in migration between lanes 2 and 7, which contained glucocerebrosidase samples recovered from cells not exposed to inhibitors of carbohydrate processing. In contrast, all of the glucocerebrosidase samples obtained from CHO cells treated with inhibitors of carbohydrate processing showed a migration shift upon treatment with Endo H, indicating the presence of an increased number of oligomannose residues and therefore exposed mannose residues on the majority of the glucocerebrosidase contained within those samples.

The presence of exposed mannose residues on glucocerebrosidase recovered from cells treated with inhibitors of carbohydrate processing was further confirmed by mass spectrometry. Figure 2 of Exhibit B of Dr. Edmunds' Declaration shows the MALDI-TOF MS spectra of the oligomannose-containing glycans released from Endo H treatment of glucocerebrosidase produced by culturing mammalian cells in the presence of the four different inhibitors of carbohydrate processing. As is evident from the signal to noise ratio in the panel labeled "Control (without inhibitor)" of Figure 2, only a very

small amount of oligomannose and hybrid type structures are released by EndoH treatment of glucocerebrosidase recovered from cells cultured without an inhibitor, and these structures are mainly Man5, Man5P and Man6P. In contrast, cells cultured in the presence of any of four different inhibitors of carbohydrate processing that act to inhibit the conversion of $Glc_3Man_9GlcNac_2$ to smaller species all produce glucocerebrosidase containing a higher number of exposed mannose residues than contained on glucocerebrosidase recovered from untreated cells.

In paragraphs 13 and 14 of his Declaration, Dr. Edmunds presents data from additional experiments demonstrating that the results achieved in CHO cells can also be produced with different mammalian cell lines. In one experiment, discussed at paragraph 13 of Dr. Edmunds' Declaration, he presents data demonstrating that HeLa cells capable of expressing human glucocerebrosidase cultured in the presence of each of the four inhibitors of carbohydrate processing produce human glucocerebrosidase having more exposed mannose that glucocerebrosidase recovered from untreated cells. Similar to what was observed with the CHO cells, the glucocerebrosidase from HeLa cell cultures treated with the inhibitors are also sensitive to Endo H, as evidenced by faster migration after Endo H treatment.

At paragraph 14 of his Declaration, Dr. Edmunds presents similar results with a different human cell line. Specifically, Dr. Edmunds presents data demonstrating that human glucocerebrosidase obtained from HEK 293 cells cultured in the presence of the four different inhibitors of carbohydrate processing also contains higher amounts of exposed mannose residues than glucocerebrosidase recovered from untreated cells.

In summary, the data set forth by Dr. Edmunds in his Declaration demonstrates that human glucocerebrosidase produced in three different mammalian cell lines (CHO, HeLa and 293) treated with four different inhibitors of carbohydrate processing that act to inhibit the conversion of $Glc_3Man_9GlcNac_2$ to smaller species, contains a higher number of exposed mannose residues than glucocerebrosidase recovered from untreated cells. Thus, Applicants have demonstrated that a skilled artisan following the teachings of the '025 application can control the glycosylation process in mammalian cell culture to produce the human glucocerebrosidase of the claims. As is not in dispute, human

glucocerebrosidase with exposed mannose residues is particularly useful for the treatment of human patients having Gaucher's disease.

In the Advisory Action, the Examiner noted that it was not clear from the data presented in the Declaration that each of the enzyme preparations produced by the various cell lines in the presence of the various inhibitors had the properties of a pharmaceutically useful glucocerebrosidase as contemplated in the specification. Specifically, the Examiner is of the view that to be pharmaceutically useful, the human glucocerebrosidase must have at least two carbohydrate moieties each having a $Man_3$-$Man_9$ structure and that such molecules would have to make up at least 50% of the glucocerebrosidase expressed.

First, applicants note that the specification contains no such absolute requirement. Rather, the portion of the specification relied upon by the Examiner for this proposition, i.e., page 26, line 27- page 27, line 4, refers to rGCR which is post-translationally modified:

> "The rGCR of this invention is useful for the therapeutic treatment of Gaucher's disease by providing a therapeutic amount of rGCR. By therapeutic amount is meant an amount of rGCR which will cause significant alleviation of clinical symptoms of Gaucher's disease. Such rGCR must be post-translationally modified, as described above, to provide a carbohydrate structure which will target to human mannose receptors. **Generally**, such rGCR has at least two carbohydrate moieties each having a $Man_3$-$Man_9$ structure, and such rGCR represents at least 50% of the rGCR provided in the therapeutic composition." (Emphasis added.)

It is clear from the text that such numbers were provided as a general guideline for the types of carbohydrate structures that would result in targeting to the mannose receptor. For example, see also page 23, lines 19-30, which state:

> "There are generally four oligosaccharide moieties in human placental GCR. Recombinant GCR having these moieties present in a $Man_3$-$Man_9$ structure has greater affinity than unglycosylated GCR for the mannose receptor in humans. The more mannose residues per oligosaccharide moiety, and the more such moieties, the greater this affinity. Recombinant GCR according to this invention has at least one such oligosaccharide with at least one exposed mannose, but preferably has two, three, or four such oligosaccharides each with a $Man_3$-$Man_9$ structure."

Moreover, Applicants also note that the presently claimed invention is described in a different section of the specification entitled "Other Embodiments." See the bottom of page 27 to page 28, which describes production of an appropriate carbohydrate structure by treating a cell expressing glucocerebrosidase during its growth with inhibitors of carbohydrate processing that act to inhibit specific steps in the conversion of $Glc_3Man_9GlcNac_2$ to smaller species.

Second, and more importantly, however, Applicants refer the Examiner to the SDS-PAGE gels and Western blots shown in Figure 1 of Exhibit B and Figures 1 and 2 of Exhibit C of Dr. Edmunds' Declaration. These figures demonstrate that the majority of the glucocerebrosidase recovered from cells treated with inhibitors of carbohydrate processing undergoes a migration shift upon treatment with Endo H, demonstrating that it contains oligomannose and hybrid type carbohydrate structures. Furthermore, the mass spectrometry spectra of the glycans released from Endo H treatment of glucocerebrosidase produced by such treated cells demonstrates that the cells cultured in the presence of deoxymannojirimyycin produced glucocerebrosidase with Man9, Man8, Man7, Man6P, Man6 and Man5 structures, cells treated with swainsonine produced glucocerebrosidase with hybrid type oligosaccharides, and cells treated with castospermine and deoxynojirimycin produced glucocerebrosidase with either Man 9 or Man9/Man8 structures (see paragraph 9 of Dr. Timothy Edmunds declaration). In contrast, glucocerebrosidase recovered from a culture of the same cells in the absence of the inhibitor contained only very small amounts of exposed mannose (mainly Man5, Man5P , Man6P). (See Dr. Edmunds' Declaration, paragraph 12.)

Thus, Dr. Edmunds' Declaration demonstrates that treatment of mammalian cells capable of expressing glucocerebrosidase with inhibitors of carbohydrate processing that act to inhibit the conversion of $Glc_3Man_9GlcNac_2$ to smaller species results in the production of glucocerebrosidase the majority of which contains exposed mannose residues. Such glucocerebrosidase would be expected to be pharmaceutically useful in that it would be appropriately targeted to the mannose receptor.

In view of the above, applicants believe that they have demonstrated that a skilled artisan would indeed recognize that Applicants were in possession of a genus of human glucocerebrosidase produced by any mammalian cell treated with any inhibitor of

carbohydrate processing that acts to inhibit the conversion of $Glc_3Man_9GlcNac_2$ to smaller species and that such glucocerebrosidase would be suitable for the treatment of human patient having Gaucher's disease. Accordingly, Applicants request that the Examiner withdraw the rejection for lack of written description.

***Rejection of claims under 35 USC § 112, First paragraph – Enablement***

Claims 60-62 were rejected under 35 USC § 112, first paragraph as failing to comply with the enablement requirement. In the January 19, 2006 Office Action and the Advisory Action, the Examiner conceded that the specification is enabling for a pharmaceutical composition suitable for the treatment of a human patient having Gaucher's disease comprising a human glucocerebrosidase produced by providing a culture of CHO cells capable of expressing said human glucocerebrosidase and treating the CHO cells with any of five identified inhibitors of carbohydrate processing. However, the Examiner contends that the specification "does not reasonably provide enablement for the broad scope of a pharmaceutical composition suitable for the treatment of a human patient having Gaucher's disease comprising a human glucocerebrosidase produced by providing a culture of any mammalian cell capable of expressing a human glucocerebrosidase and treating the cell with any inhibitor [of] carbohydrate processing that acts to inhibit the conversion of $Glc_3Man_9GlcNac_2$ to smaller species." According to the Advisory Action, Applicants' contention that the human glucocerebrosidase of the claims refers to the polypeptide known in the art at the time of filing is persuasive. However, Applicants' arguments with regard to predictability of glycosylation in cell culture were not found persuasive because they were based on Dr. Edmunds' Declaration, which was not entered, and in any event, the Examiner is of the view that the Declaration did not establish that each of the enzyme preparations produced by the various cell lines in the presence of the various inhibitors had the properties of a pharmaceutically useful glucocerebrosidase, as further detailed in connection with the written description rejection, above.

Evidence demonstrating the relative predictability of glycosylation in cell culture using the teachings of the '025 application has been discussed above and in the Declaration of Dr. Edmunds. Applicants' data demonstrate that treatment of three

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different mammalian cell cultures (CHO, Hela and 293) capable of expressing human glucocerebrosidase with four different inhibitors of carbohydrate processing results in the production of human glucocerebrosidase the majority of which contains a higher number of exposed mannose residues than does human glucocerebrosidase recovered from untreated cells. Moreover, Dr. Edmunds concludes in paragraph 15 that in his opinion, the data set forth in the Declaration are sufficiently strong and complete that they are fairly extrapolated to other mammalian cell types and other inhibitors of carbohydrate processing that act to inhibit the conversion of $Glc_3Man_9GlcNac_2$ to smaller species.

In sum, Applicants have demonstrated that the invention as now claimed is fully enabled, because a skilled artisan following the teachings of the specification could readily practice the claimed invention with a variety of mammalian cells types and a variety of inhibitors of carbohydrate processing.

## CONCLUSION

In view of the amendments to the claims and the foregoing remarks, Applicants request that the rejections be reconsidered and withdrawn. In addition, if the product claims are found allowable, Applicants request that the withdrawn process claims be rejoined in accordance with the provisions of MPEP § 821.04, and that claims drawn to non-elected species be considered. If the Examiner believes that a conversation with Applicants' attorney would be helpful in expediting prosecution of this application, he is invited to call the undersigned at the number provided below.

Respectfully submitted,

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